

Extended-spectrum beta-lactamase-producing *Escherichia coli* harbouring *mcr-1* gene isolated from pigs in South Africa

To the Editor: Extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) produce enzymes that confer resistance to penicillins, cephalosporins and monobactams.^[1] ESBL-PE represent a global concern in humans, in animals and in the environment and have therefore been recognised as pathogens of critical priority.^[2] Of additional concern is the emergence of the plasmid-borne colistin resistance (*mcr-1*) genes that encode for resistance to polymyxins, the antibiotics of last resort for treatment of serious difficult-to-treat infections.^[3] They have both been detected in developed and developing countries, and food animals have been recognised as their principal reservoir.^[4]

Nasal and rectal swabs were collected from 432 pigs during a multicentre study carried out from March to October 2016 in five abattoirs in Cameroon (*n*=3) and South Africa (SA) (*n*=2). Samples were screened on a selective medium and putative ESBL-PE were tested for their antimicrobial susceptibility using the Vitek 2 System and Vitek 2 Gram Negative Susceptibility card (AST-N255) (bioMérieux, France). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline,^[5] with the exception of colistin, amoxicillin plus clavulanic acid, piperacillin plus tazobactam and amikacin, which were based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.^[6] Closely related isolates underwent whole-genome sequencing (WGS) analysis on an Illumina MiSeq platform (Illumina Inc., USA) with 100 × coverage. ResFinder,^[7] VirulenceFinder^[8] and PlasmidFinder^[9] were used to identify resistance genes, virulence factors and plasmids, respectively. The multi-locus sequence type was also determined from WGS data.

One of the ESBL-producing *Escherichia coli* isolated, PN256E8 (GenBank accession no. QJRZ00000000), showed resistance to several beta-lactam and non-beta-lactam antibiotics, including colistin. This resistance phenotype was corroborated by the identification of the *bla*_{TEM-1B} and *bla*_{CTX-M-55} genes through WGS, which also evidenced the colistin (*mcr-1*)-resistant gene (Table 1). The isolate PN256E8 was ascribed to the sequence type (ST) 446 and phylogenetic group A. It further harboured two virulence factors

and two replicons along with three plasmid incompatibility groups, IncHI2 (ST-3-like), IncHI2A (ST-3-like) and IncN (ST-1).

To the best of our knowledge, this is the first report on the presence of an *mcr-1* encoding colistin resistance in ESBL-*E. coli* ST446 isolated from a pig abattoir in SA. The dissemination of *mcr-1*-producing *E. coli* strains in food animals and humans has been reported worldwide. In SA, a 79% prevalence of *mcr-1* in colistin-resistant cultures of *E. coli* was reported in a nationwide surveillance programme of poultry settings. The *mcr-1* gene was detected among clinical *E. coli* isolates of hospitalised patients (*n*=3) and outpatients (*n*=6) not previously exposed to colistin.^[10] Furthermore, in the Western Cape region, 83% of clinical colistin-resistant Enterobacteriaceae harboured the *mcr-1* gene.^[3] The concomitant presence of ESBL and *mcr-1* genes in *E. coli* ST 446 is an important food safety and public health concern, as these resistances could be transferred from commensal to pathogenic bacteria prevailing in the food chain and subsequently disseminate to communities and/or healthcare settings. Increased surveillance of ESBL producers for colistin resistance is essential to monitor their acquisition and spread. Efforts to ensure rational antibiotic use in agriculture are vital to preserve antibiotics for future generations.

Acknowledgements. We thank the abattoir owners/co-ordinators for granting access to their structures and for their hospitality.

Author contributions. LLF co-conceptualised the study, undertook sample collection and microbiological laboratory and data analyses, prepared tables, interpreted results, contributed to bioinformatics analysis, and drafted the manuscript. RCF undertook sample collection and microbiological laboratory analyses, contributed to bioinformatics analysis and vetted the results. MA undertook bioinformatics analyses. AI performed whole-genome sequencing analysis. SYE co-conceptualised the study and undertook critical revision of the manuscript. All authors read and approved the final manuscript.

Funding. LLF and RCF are funded by the Antimicrobial Research Unit and College of Health Sciences of the University of KwaZulu-Natal. The National Research Foundation (NRF) funded this study through the NRF Incentive Funding for Rated Researchers (grant no. 85595), the NRF Competitive Grant for Rated Researchers (grant no. 106063) and the DST/NRF South African Research Chair in Antibiotic

Table 1. Antimicrobial susceptibility results of selected beta-lactam and non-beta-lactam antibiotics

Isolate name	Beta-lactam antibiotics										Non-beta-lactam antibiotics										Virulence genes	Plasmids
	AMP	AMC	TZP	CXM	CTX	CAZ	FEP	ETP	MEM	IMP	GEN	AN	CIP	TGC	FT	CS	SXT	TMP/	Antibiotic resistance genes			
PN256E8	R	S	S	R	R	R	R	S	S	S	R	R	S	S	S	R	R	R	<i>bla</i> _{TEM-1B} , <i>bla</i> _{CTX-M-55} , <i>aacA4</i> , <i>aadA5</i> , <i>aac(6')</i> , <i>lb-cr</i> , <i>oqxB</i> , <i>fosA</i> , <i>tet(A)</i> , <i>floR</i> , <i>drfA7</i> , <i>drfA17</i> , <i>su12</i> , <i>mcr-1</i>	Col(MG828) RepA, IncHI2 (ST-3-like), IncHI2A (ST-3-like), IncN (ST-1)		

AMP = ampicillin; AMC = amoxicillin plus clavulanic acid; TZP = piperacillin plus tazobactam; CXM = cefuroxime; CTX = cefotaxime; CAZ = ceftazidime; FEP = cefepime; ETP = eripenem; MEM = meropenem; IMP = imipenem; GEN = gentamicin; AN = amikacin; CIP = ciprofloxacin; TGC = tigecycline; FT = nitrofurantoin; CS = colistin; TMP/SXT = trimethoprim plus sulfamethoxazole; R = resistant; S = susceptible; *aacA4* = EAST-1 heat stable toxin; *gad* = glutamate decarboxylase.

Resistance and One Health (grant no. 98342) awarded to SYE. The South African Medical Research Council also funded the study through the Self-Initiated Researcher Grant awarded to SYE. Any opinions, findings and conclusions, or recommendations expressed in this review are those of the authors, and therefore do not represent the official position of the funders. The funders had no role in the study design, preparation of the manuscript or the decision to submit the work for publication.

Conflicts of interest. SYE is a member of the Global Respiratory Infection Partnership, sponsored by an unrestricted educational grant from Reckitt Benckiser, UK. All the other authors declare that they have no competing financial interests.

Luria Leslie Founou

Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa; and Department of Food Safety and Environmental Microbiology, Centre of Expertise and Biological Diagnostic of Cameroon, Yaoundé, Cameroon
luriafounou@gmail.com

Raspail Carrel Founou

Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa; and Department of Clinical Microbiology, Centre of Expertise and Biological Diagnostic of Cameroon, Yaoundé, Cameroon

Mushal Allam, Arshad Ismail

Sequencing Core Facility, National Health Laboratory Service, Johannesburg, South Africa

Sabiha Yusuf Essack

Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

1. Perovic O, Singh-Moodley A, Duse A, et al. National sentinel site surveillance for antimicrobial resistance in *Klebsiella pneumoniae* isolates in South Africa, 2010 - 2012. *S Afr Med J* 2014;104(8):563-568. <https://doi.org/10.7196/SAMJ.7617>
2. World Health Organization. Global Priority List of Antibiotic Resistant Bacteria to Guide Research, Discoveries and Development of New Antibiotics. Geneva: WHO, 2017.
3. Newton-Foot M, Snyman Y, Maloba MRB, Whitelaw AC. Plasmid-mediated *mcr-1* colistin resistance in *Escherichia coli* and *Klebsiella* spp. clinical isolates from the Western Cape region of South Africa. *Antimicrob Resist Infect Control* 2017;6:78. <https://doi.org/10.1186/s13756-017-0234-8>
4. Founou LL, Founou RC, Essack SY. Antibiotic resistance in the food chain: A developing country-perspective. *Front Microbiol* 2016;7(1881). <https://doi.org/10.3389/fmicb.2016.01881>
5. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Six Supplement M100S. Wayne, Penn.: CLSI, 2016.
6. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 6.0, 2016.
7. Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012;67(11):2640-2644. <https://doi.org/10.1093/jac/dks261>
8. Joensen KG, Scheutz F, Lund O, et al. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 2014;52(5):1501-1510. <https://doi.org/10.1128/JCM.03617-13>
9. Carattoli A, Zankari E, García-Fernández A, et al. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014;58(7):3895-3903. <https://doi.org/10.1128/AAC.02412-14>
10. Coetsee J, Corcoran C, Prentice E, et al. Emergence of plasmid-mediated colistin resistance (MCR-1) among *Escherichia coli* isolated from South African patients. *S Afr Med J* 2016;106(5):449-450. <https://doi.org/10.7196/SAMJ.2016.v106i5.10710>

S Afr Med J 2018;108(10):796-797. DOI:10.7196/SAMJ.2018.v108i10.13508